REMARKS/ARGUMENTS

Reconsideration and withdrawal of the rejections of the present application are respectfully requested in view of the amendments to the claims and remarks presented herewith, which place the application into condition for allowance, or in better condition for appeal.

Status of the Claims and Formal Matters

Claims 1-3, 6-15, 22, 24-29, and 32-34 are currently pending in this application. By this paper, Claims 1, 22, and 34 have been amended, without prejudice, and solely to expedite prosecution pursuant to the U.S. Patent and Trademark Office Business Goals (65 Fed. Reg. 54604 (September 8, 2000)). Claim 26 has been cancelled. Applicants respectfully assert the right to reclaim withdrawn or cancelled subject matter in co-pending applications. No new matter has been introduced by these amendments. Support for the amendments can be found throughout the specification as originally filed, in particular, original Claim 26 and page 33, lines 10-30 to page 34, lines 1-6.

Rejections under 35 U.S.C. §103(a)

Claims 1-3, 6-14, 22, 24, 25, 32, and 33 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Griffiths (U.S. Patent Application Publication No. 20020119459; herein "Griffiths") in view of Andreadis et al., ((2000) Nucleic Acids Res. 28:e5, pp. i-viii; "Andreadis") and further in view of Wangh et al. (U.S. Patent Application Publication No. 20040053254; "Wangh"). The Office Action contends that it allegedly would have been *prima facie* obvious to combine the method of Griffiths for amplifying nucleic acids in a microcapsule such as a water-in-oil emulsion on the surface of a bead contained within the emulsion with the methods of Andreadis and Wangh for performing asymmetric PCR using two populations of a first primer, one attached to a solid surface and one in lower concentration in solution, since the primer in solution can allegedly be used to exhaustion to perform the initial rounds of amplification on the target nucleic acid in combination with the second primer, while the resulting extension products can be further amplified in the solid phase to generate products

immobilized on the bead. The Office Action further argues that the asymmetric PCR methods of Andreadis and Wangh are adaptable to performing in microcapsules such as water-in-oil emulsion droplets, since such microcapsules are sufficiently large to accommodate any conditions or reaction requirements for amplification reactions such as PCR.

Upon entry of the present response, Applicants have amended claims 1, 22, and 34 to include the limitation of extending at least 100,000 bead bound complementary strands. Griffiths relates to methods of *in vitro* sorting of genes and their respective gene products within microcapsules. Applicants note that Griffiths does not teach or disclose "at least 100,000 bead bound complementary strands" as claimed.

Andreadis does not cure the deficiencies of Griffiths. Andreadis does not teach or disclose the use of microreactors comprised of water-in-oil emulsions, nor does Andreadis teach or disclose at least 100,000 bead bound complementary strands. For the same reason, Wangh also fails to cure the defects of Griffiths. Wangh, like Andreadis, is silent regarding aqueous microreactors comprised of water-in-oil emulsions and fails to teach or disclose "at least 100,000 bead bound complementary strands" as claimed. This is further supported by the Office Action which on page 13 states "Neither Griffiths nor Andreadis nor Wangh teach a method for amplifying one or more nucleic acids wherein more than 10,000 or at least 1,000,000 amplification copies of each target nucleic acid molecule are bound to each bead".

For at least all of the foregoing reasons, Applicants respectfully contend that *prima facie* obviousness under §103(a) has not been established, particularly where none of the cited references teach or disclose all of the instant claim limitations with a reasonable expectation of success. Applicants respectfully request reconsideration and withdrawal of the §103(a) rejection.

Claims 15 and 26-29 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Griffiths in view of Andreadis and Wangh and further in view of Jurinke et al. (U.S. Patent No. 6,303,309; "Jurinke"). None of Griffiths, Andreadis, or Wangh teach a method for amplifying one or more nucleic acids wherein at least 100,000 amplification copies of each target nucleic acid molecule are bound to each bead, nor do these references teach a method for

amplifying one or more nucleic acids wherein the beads are Sepharose beads. Jurinke allegedly teaches a method of purification of biotin-labeled PCR products by complexing the products to a solid support containing a biotin-binding compound such as streptavidin immobilized on the surface. Jurinke also allegedly teaches immobilization of 100 pmol biotinylated oligonucleotides to 50 µl (~40 million) streptavidin-coated magnetic beads, which represents about 1 million molecules bound per bead. The Office Action contends that it would allegedly have been obvious to combine the methods of Griffiths, Andreadis, and Wangh for amplifying nucleic acids on a bead within a microcapsule such as a water-in-oil emulsion using non-symmetric PCR with that of Jurinke for purification of PCR products using solid supports, such as magnetic or Sepharose beads, since the use of such beads allegedly allows further purification and extensive washing to remove all excess reaction components prior to final recovery of the PCR product. Applicants respectfully traverse.

Applicants respectfully assert that the Office Action has not established *prima facie* obviousness under §103, because, as argued elsewhere in this Response, the combination of Griffiths, Andreadis, and Wangh do not teach or disclose all of the instant claim limitations with a reasonable expectation of success. Jurinke does not cure these deficiencies because Jurinke fails to teach or disclose the extension of at least 100,000 bead bound strands complementary to a template nucleic acid as claimed. The Office Action attempts to infer the inherency of a result produced from certain reaction conditions described by Jurinke, specifically the result of a known number of immobilized nucleic acid molecules that is neither described by Jurinke nor necessarily a true result of the described reaction conditions.

For example, at page 13, the Office Action argues, "Jurinke also teaches immobilization of 100 pmol biotinylated oligodeoxynucleotide to 50 µl (***~40 million***) streptavidin-coated magnetic beads (column 8, 12, lines 13-26), which ***represents*** about ***1 million molecules bound per bead***" (Emphasis added by Applicants). Applicants respectfully assert that the argument presented in the Office Action takes the description in Jurinke out of context and adds specific details not described by Jurinke in an attempt to support the argument. For instance, there is no description in Jurinke that the described 50 µl of beads have about 40 million individual beads

and the Office Action fails to explain how the 40 million number was derived. In addition to the lack of description to the number of beads, there is no described dimension of the beads in the example used to support the argument or in the passage in col. 8 of Jurinke cited by the Office Action. Those of ordinary skill would clearly appreciate that the dimension of a bead has a direct relationship to the volume it occupies and thus determines the number of beads a specific volume could hold.

In fact, the argument presented in the Office Action is based on an ***assumption*** that 50 µl of beads must have 40 million or fewer beads, that essentially every molecule added to the reaction binds to the beads, and that such binding occurs in equal concentration on the individual beads. However, these assumptions do not account for any experimental artifact and contradict the context in which Jurinke describes the intention of the experiments. This is clear because Jurinke ***never*** tests the number of molecules bound to a bead, instead Jurinke uses MALDI-TOF to demonstrate that ammonium hydroxide is capable of dissociating biotinylated nucleic acids immobilized on streptavidin supports and illustrates that the size of dissociated molecule is correct. Thus, Jurinke is only concerned that a detectable number of biotinylated nucleic acids were immobilized rather than an estimation of that number. In the example used to support the arguments in the office action, Jurinke states at col. 12, lines 41-43:

> "The spectrum demonstrates that a biotinylated oligodeoxynucleotide is removed from the beads"; and
>
> (col. 12, lines 46-48) "This Example demonstrates that biotinylated DNA can be recovered from a biotin-streptavidin complex using ammonium hydroxide at elevated temperatures"

Importantly, one of ordinary skill would interpret the example of Jurinke upon which the Office Action's argument lies to describe reaction conditions used to immobilize detectable amounts of nucleic acid to the beads. In fact, in the example, Jurinke describes exposing the beads to the ammonium hydroxide (i.e., to dissociate the biotin-streptavidin bond) after immobilization without an intervening detection step (i.e. only an intervening wash step). It is also important to note that the beads were not used in the detection step as described by Jurinke,

rather the dissociated nucleic acids were collected as a supernatant and processed for analysis by MALDI TOF. For example, col. 12 lines 13-26 of Jurinke state:

> "For this example, 100 pmol biotinylated oligodeoxynucleotide (20 mer), 5'd(bio-AGCTCTATATCGGGAAGCCT)3' (SEQ ID NO:1), were immobilized on 50µl streptavidin Dynabeads M-280. The ***beads were prepared*** according to the instructions of the manufacturer. The beads were finally resuspended in 50 µl of B/W-buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 2 M NaCl). The ***oligodeoxynucleotide was added*** in a volume of 1 µl and the reaction mixture was ***incubated for 30 minutes*** at ambient temperature. ***After immobilization***, the beads were washed twice with 100 µl of ammonium citrate buffer (0.07 M). The beads were washed once with ultrapure water, ***25 µl of a 25% solution of ammonium hydroxide were added***, and the beads were carefully resuspended. The suspension was incubated at 60° C. for 10 minutes, and the ***beads were separated*** from the solution using a magnetic particle collector (Dynal, Hamburg, Germany). The ***supernatant*** was saved and the procedure was repeated once. Both ***supernatants were collected*** in a single tube. The ***solution*** was lyophilized for 30 minutes, and ***redissolved*** in 4 || 1 of ultrapure water. From this ***solution*** 0.5 µl were ***analyzed with MALDI-TOF*** mass spectrometry (FIG. 4) as described in example 2, step 4." (Emphasis added by Applicants)

In other words, one of ordinary skill would appreciate that the reaction conditions described by Jurinke consisted of 1 µl of 100 pmol concentration biotinylated nucleic acid was added to 50 µl of beads suspended in 50 µl of buffer and incubated. One of ordinary skill would further appreciate that the actual number of nucleic acids molecules immobilized on each bead depends on a number of factors such as the concentration of solutions, available and accessibility of immobilization sites per bead, temperature, incubation time, etc. Applicants respectfully assert that given the description of Jurinke, there is no way of knowing how many beads were employed, or how many immobilized nucleic acid molecules were present on each bead. Also, the detection step described by Jurinke used a sample of dissociated nucleic acid molecules collected and ***combined from all beads*** present in the reaction and ***only produced an estimation of the size of the nucleic acids*** to verify their presence or absence in the sample.

Therefore, Applicants respectfully reiterate that the Office Action fails to support the argument based upon actual description in Jurinke. Applicants also assert that the argument that the number of immobilized copies would be an inherent result of the described reaction

conditions is without merit and cannot be supported. Therefore, Applicants respectfully request that the rejection be withdrawn.

In addition, as Applicants state above claims 1 and 22 as amended include the step of sequencing the bead bound complementary strands which Applicants respectfully assert clarifies the importance of the claimed number of immobilized copies. For example, the amplification and immobilization of many copies of the template (or complement thereof) is important in many embodiments of sequencing technology to effectively detect and enable accurate recognition of signals generated in the sequencing process. For instance, for many sequencing methods 100,000 copies of a template provide sufficient signal strength generated from at least a substantial proportion of the 100,000 copies where the signal is proportionally greater than if it was a single incorporation event. In the example of pyrosequencing, a single incorporation event results in the release of a single photon of light which is below the threshold of many detection devices, particularly those constructed to detect from a wide field of view to detect many reaction events in parallel. Fluorescent labels also suffer from similar drawbacks in the ability to produce enough emitted light from a single or small number of incorporation events.

Claim 34 was rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Griffiths in view of Andreadis and further in view of Nakano et al. ((2003) J. Biotech. 102: 117-124; "Nakano"). The Office Action contends that it would allegedly have been obvious to combine the methods of Griffiths and Andreadis for amplifying nucleic acids on a bead within a microcapsule such as a water-in-oil emulsion using asymmetric PCR with that of Nakano, since Nakano allegedly teaches a method for amplifying multiple nucleic acids in water-in-oil emulsions that is adaptable to the methods of Griffiths and Andreadis using a bead to bind amplification products in solution. Applicants respectfully traverse.

As argued elsewhere in this Response, the combination of Griffiths and Andreadis fail to establish *prima facie* obviousness under §103(a), because neither of these references teach or disclose all of the instant claim limitations, particularly "at least 100,000 bead bound complementary strands," with a reasonable expectation of success. Nakano also fails to cure the defects of Griffiths and Andreadis.

Nakano describes the use of polymerase chain reaction to amplify a single template DNA molecule in a water-in-oil emulsion. The Nakano method consists of emulsification of template DNA in bulk oil phase, followed by PCR amplification. Nakano at page 120 discloses that "[t]he droplet size in the W/O emulsion ranged from 2 to 10 μm in diameter." There is no other teaching or disclosure in Nakano of water-in-oil emulsions that are larger than 10 μm. Nakano further discloses at page 121, right column that "[w]hen a miniaturized reactor, having a volume of 280 nl, permitted PCR amplification from a single template DNA, the effective concentration was around 5.8×10^{-18} M." Nakano does not disclose the actual numbers or concentrations of PCR product present within the water-in-oil emulsion droplets. However, it is very likely that, in view of the small size of the emulsion droplets described in Nakano, these droplets are incapable of supporting "at least 100,000 bead bound complementary strands" as claimed. There is no teaching or disclosure in Nakano of beads disposed within microcapsules as claimed.

Nakano does not cure the deficiencies of Griffiths and Andreadis. This combination of references fails to establish *prima facie* obviousness under §103(a), because it fails to disclose all of the instant claim limitations with a reasonable expectation of success. None of Griffiths, Andreadis, or Nakano teach "at least 100,000 bead bound complementary strands" as claimed in amended Claim 34, nor is there any teaching in any of these references that such a modification was contemplated or considered. Those of ordinary skill in the art would be provided with no guidance or reasonable expectation of success in practicing the presently claimed invention in view of the complete lack of disclosure in Griffiths, Andreadis, and Nakano.

For at least all of the foregoing reasons, Applicants respectfully request reconsideration and withdrawal of the §103(a) rejection over the combination of Griffiths, Andreadis, and Nakano.

CONCLUSION

Favorable action on the merits is respectfully requested. If any discussion regarding this Response is desired, the Examiner is respectfully urged to contact the undersigned at the number given below, and is assured of full cooperation in progressing the application to allowance.

Applicants believe no additional fees are due with this Amendment and Response; however, if any additional fees are required or if any funds are due, the USPTO is authorized to charge or credit Deposit Account Number **50-0311**, Customer Number: **35437**, Reference Number: **21465-508001US**.

Respectfully submitted,

Dated: July 1, 2009

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